WELLCOME
LIBRA
Coll. WelMO
Coll. pam
No WL100
1900
D130

[*Reprinted from the Journal of Physiology.*
Vol. XXV. No. 3, *February* 2, 1900.]

22501278658

ON SOME NUMERICAL COMPARISONS OF THE CENTRIPETAL AND CENTRIFUGAL MEDULLATED NERVE-FIBRES ARISING IN THE SPINAL GANGLIA OF THE MAMMAL.

By H. H. DALE, B.A.
Scholar of Trinity College, Cambridge, Coutts Trotter Student.
(Plate II.)

WELLCOME LIBRARY INSTITUTE

The investigations recorded in this paper were made with two objects in view, viz., in the first place to determine the relative number of medullated fibres centrally and proximally of the spinal ganglion, and in the second place to determine the relative number of the fibres of any given size in the same situations. An exact knowledge of these points would go far towards deciding whether any fibres end free in the spinal ganglion; whether either of the cell processes divides in the ganglion, or soon after its exit from the same; and whether the central and peripheral processes of any or of all the spinal ganglion cells are unequal in diameter.

The relative number of fibres on the proximal and distal sides of the ganglion.

Historical. Investigations on this point are by no means new. I may pass over the early observations of Wagner, Robin, Bidder, Kölliker and others, which bear more or less upon the question, merely noting that the results differed in many points, but that the balance of opinion seemed in favour of an excess of fibres on the peripheral side. Of more direct interest in this connexion are the actual countings of the fibres which have been made in the frog and in mammals. Holl[1] and Stiénon[2] both made countings in sections of the trunk and roots of the frog and the mammal, and Birge[3] made similar countings in the frog only. All these observers found, in most cases, a practical identity in the numbers, but it should be noted that, when a small difference was detected, it was in almost every case on the side of the trunk. These small differences they all attribute to

experimental error. In more recent years countings have been made by Gaule and Lewin[4] on the sacral nerves of the rabbit, and by Bühle[5] and Hardesty[6] in the frog. These observers come to the conclusion that there is a considerable excess (up to 38 %) of fibres on the peripheral side, and they attribute the difference to division of the peripheral processes of the ganglion cells, to the failure of some of the ganglion cells to contribute medullated processes to the posterior root, and to the presence of medullated post-ganglionic sympathetic fibres ending in the spinal ganglion. Hardesty, whose paper is of especial interest to me in that he has used the photographic method which I had myself adopted, not only finds a difference in the countings proximally and distally to the ganglion, but also a progressive decrease in the number of the fibres of the ventral root in passing from the spinal cord towards the ganglion; a progressive increase in the same direction in the dorsal root; and a similar decrease in the number of fibres in the trunk in passing from the ganglion to the periphery.

It will be seen that the results are not very consistent, but the tendency is to show that a spinal nerve has rather more medullated fibres in the trunk near the ganglion than in the roots.

Methods. My own experiments have been made chiefly on the coccygeal nerves of the cat. These were chosen as being convenient for dissection and as containing a workably small number of fibres. They have the further advantage, which directed Gaule's choice of sacral nerves, of presenting a considerable stretch of trunk inside the vertebral canal, the danger of losing small twigs in dissection being thus eliminated. Two thoracic nerves from the cat and one lumbar nerve from the rat were also counted.

Various methods of preservation and staining were tried, including the use of formalin, followed by osmic acid. This preserves the fibres with a minimum of shrinkage, but the medulla, when so preserved, has a tendency to split in such a manner as to show a radiate structure in transverse section—an appearance which renders the section practically useless for the photographic method which I employed. The best results were obtained by directly immersing the nerves in 1 % osmic acid. The coccygeal roots were carefully exposed by removing the neural arches of the vertebræ and opening the dura mater longitudinally in the coccygeal region. The first coccygeal nerve was identified, usually by counting down from the second cervical, and was cut through close to its exit from the spinal canal. The mass of roots was gently lifted by seizing the cut edge of dura mater with forceps

opposite the first coccygeal ganglion. The dura mater was cut round the ganglion, which could now be lifted gently, the roots attached to it being separated from the rest without injury or stretching, and finally cut across as near the cord as necessary. The preparation was lifted out by the fragment of dura mater attached to the ganglion and spread on a piece of stiff paper in such a way that the trunk lay in contact with the paper and the roots were bent over to lie on the trunk. It was unnecessary in this procedure to touch either the trunk or the roots, and both could be gently extended to the same degree by pulling the fragment of dura mater. A small oblong of paper with the preparation on it was cut off from the sheet and put directly into 1% osmic acid. When the nerve had become stiffened in the required position it was detached from the paper and returned to the osmic acid for 24 hrs. The same method was used for preparation of any coccygeal nerve and roots, and for the lumbar roots of the rat. In the latter case, as in the thoracic roots of the cat, the dorsal branches of the nerves, which arise practically from the ganglia, were carefully dissected out and laid parallel to the main trunk.

After 24 hours in osmic acid, the preparations were washed for 1—2 hours in running water and passed through graduated alcohols to absolute alcohol, absolute and xylol, xylol, and paraffin melting at 52° C. In some cases cedar oil was used, but this, though it produces less shrinkage than xylol, has a dangerous property of dissolving the osmium-impregnated medulla, which becomes evident very shortly after the preparation has become thoroughly penetrated with the oil. Xylol, in the cold, seems to have no such tendency.

Sections cut with six teeth of the Cambridge Rocking Microtome—i.e. about 4μ thick—were found to be of about the requisite thickness. Thinner sections were too pale to give sharp negatives.

For some time I tried to obtain reliable countings and measurements by careful drawings of the sections with Abbe's drawing apparatus. I found, however, that the method was not reliable, as it was impossible to draw the trunk under a sufficiently high power without frequent shiftings, and even the use of a mechanical stage could not prevent a certain degree of displacement and consequent error. In one case, where such shifting was unnecessary, I used the drawings so made for purposes of measurement and obtained instructive results. All my other results, however, have been obtained with the help of photography. By use of a moderately low objective (a Reichert 8 mm. gave good results) and a long-bellows camera, even the largest

of my sections could be brought into a moderately flat field and countable photographs obtained. In order to get results at all reliable and consistent I found it necessary to compare the photograph, during counting, with the section, which was viewed with Obj. $\frac{1}{6}$ Oc. 2. I found it surprisingly easy to identify any particular fibre or group of fibres, and the photographs were compared practically fibre by fibre with the sections. The photograph of each fibre, as counted, was marked in the middle with an ink-spot.

When this method of comparison with the section had been adopted, my results, which had before been uncertain, at once became consistent and unmistakable. When the photograph was counted without reference to the section I had frequently observed a considerable excess of fibres in the trunk. In such cases reference to the section always showed that a number of small fibres in the roots, through being slightly out of focus, were represented in the photographs only by almost imperceptible markings. This tendency to fall slightly out of focus affected chiefly the fibres of smallest size (2μ and less in diameter), which in the coccygeal region occur in considerable numbers in the dorsal roots. Even in the sharpest photographs of the most carefully mounted sections some additional small fibres, chiefly in the dorsal root, were always discovered by subsequent comparison with the section. A few such fibres, but by no means so many, were usually discovered in the trunk. I imagine that, in the case of the trunk, the intervening connective tissue assists in retaining the small fibres in the same plane as the rest of the section.

Results of counting.

		Ventral root	Dorsal root	Total in roots	Trunk	Excess in trunk	% excess
Cat A.	2nd coccygeal	577	1522	2099	2116	17	·8
Cat B.	4th ,,	759	1003	1762	1765	3	·17
,,	6th ,,	546	840	1384	1390	4	·3
,,	7th ,,	513	689	1202	1217	15	1·25
Cat D.	1st ,,	654	1751	2405	2427	22	·9
,,	2nd ,, (left)	633	1857	2490	2504	14	·57
,,	,, ,, (right)	643	1628	2271	2292	22	·92
,,	4th cocc. (near ganglion)	850	914	1764	1772	8	·46
,,	,, (some mm. from ganglion)	850	917	1767	1772	5	·28
					Average % excess in coccygeal nerves		·63
Cat E.	3rd thoracic	3032	3050	6082	6121	39	·61
,,	6th ,,	2915	2268	5183	5208	25	·48
Rat.	5th lumbar	1243	2804	4047	4065	18	·45

No preparations were used for counting which appeared in any way defective. In several cases, for no known reason, the sections of many fibres were much distorted. No such preparations were used.

With regard to the results shown in the above table I may first deal with the point raised by Hardesty (*vide sup.*), who described an alteration in the number of fibres in the course of the nerve-roots, and in the course of the trunk between the ganglion and the separation of the first branch. Two sets of countings were made on the 4th coccygeal nerve (Cat D) to test this statement. Photographs were made of sections of the roots and of the trunk taken near the ganglion, and again of sections taken at some distance from the ganglion on either side, the distance, which was the same in both cases, being such that the trunk had already split into dorsal and ventral divisions. It will be seen that the same number of fibres was present in the ventral root near the spinal cord and near the spinal ganglion; that the same number of fibres was present in the trunk near the ganglion and near the point where the dorsal branch is given off; and that in the dorsal root three more fibres were found in the section near the cord than in that near the ganglion—a trifling difference, and in the opposite direction to that indicated by Hardesty. I am in consequence unable to agree with Hardesty's conclusions on this point.

Turning now to the chief results of the observation we see that there is constantly a slight excess of fibres in the trunk over the sum in the roots, the number varying from 3 to 39. On the whole the magnitude of the excess corresponds roughly to the number of fibres in the nerve, the average difference being about ·5 %. In many cases the excess is so small that it could be quite well attributed to experimental error, but the fact that it is always on the same side leads me to believe that the result has some real significance. There is no essential difference between the thoracic, lumbar, and coccygeal nerves in this respect: in other words, the presence of fibres for the sympathetic system or for the limbs does not affect the result.

The excess of nerve-fibres peripherally of the spinal ganglion is, I think, in all probability, due to the presence in this region of medullated fibres passing to the nerve from the grey ramus and ending in the ganglion. Whether these fibres are vaso-motor fibres for the vessels of the ganglion, or whether they end in connection with the nerve-cells, in the manner described by Ramon y Cajal[7] and by Dogiel[8] my observations leave undecided. In support of this view, it may be noted that the peripheral excess which I observed in the coccygeal region

corresponds fairly with the number of the medullated fibres described by Langley[9] in the grey rami of that region, and that the excess on the peripheral side is apparently due to small fibres (6μ and less in diameter) such as are found in the grey rami.

II. The size of the fibres in the nerve-roots and in the trunk.

Historical. Various observers have stated that of the two branches given off by the process of a spinal ganglion cell the central branch, *i.e.* that running to the posterior root, is generally smaller than the peripheral branch. Wagner[10] found it so in Torpedo, Freud[11] in Petromyzon, Lenhossek[12] in the frog, Van Gehuchten[13], Cajal[7] and Dogiel[8] in the mammal, and Cajal in the bird.

On the other hand an old observation of Bidder and Volkmann[14], made indeed by a rough method of estimation, pointed to a greatly increased proportion of small fibres in the trunk.

Measurements and countings to determine the size of the fibres were made in three cases, viz. the 1st, 4th and 7th coccygeal nerves of the cat.

Methods. The first measurement was made from camera lucida drawings of sections of the 4th coccygeal roots and nerve of a rather small cat. The preparation was made with formalin, followed by osmic acid—a method which preserves the fibres in a well-rounded condition but which does not give sufficient black-and-white contrast for successful photography. The trunk in this section was split naturally into several small and well-marked divisions, so that the difficulty of shifting the section accurately was eliminated. A drawing of a micrometer scale was made with the same magnification as the drawings of the sections, and the fibres were measured with reference to this scale by compasses. The other measurements were made on enlargements from the photographs of the 7th coccygeal nerve of Cat B, and the 1st coccygeal nerve of Cat D. The photographs of the trunk, of the roots, and of a micrometer scale were in each case carefully made with the same magnification, and the enlargements from the several negatives of each set were made at one time, great care being taken to keep the adjustment of the enlarging camera unaltered. The measurements were made in the same way as on the drawing, and in all three cases the fibres were arranged in classes differing from one another by 2μ in diameter.

Cat C. 4th coccygeal.

Diameters	Ventral root	Dorsal root	Total roots		Trunk	
16μ & upwards	3	13	16		15	
14μ—16μ	19	40	59	242	37	199
12μ—14μ	51	116	167		147	
10μ—12μ	27	154	181		184	
8μ—10μ	19	155	174	527	207	584
6μ—8μ	19	153	172		193	
Less than 6μ	171	574	745		735	
			1514		1518	
				Grey ramus	30	
					1548	

Cat B. 7th coccygeal.

14μ & upwards	4	5	9	34	5	25
12μ—14μ	12	13	25		20	
10μ—12μ	50	61	111		87	
8μ—10μ	92	157	249	622	270	621
6μ—8μ	93	169	262		264	
Under 6μ	262	284	546		571	
			1202		1217	

Cat D. 1st coccygeal.

Diameters	Ventral root	Dorsal root	Total roots		Trunk	
18μ & upwards	3	1	4		3	
16μ—18μ	39	19	58	313	21	210
14μ—16μ	61	41	102		75	
12μ—14μ	71	78	149		111	
10μ—12μ	69	231	300		301	
8μ—10μ	53	152	205	838	262	951
6μ—8μ	54	279	333		388	
Less than 6μ	304	950	1254		1266	
			2405		2427	

With regard to the first counting, viz. that of the 4th coccygeal nerve; it may be noted that, in this section, the grey ramus was distinguishable as one large and three small bundles in the trunk. Among the masses of non-medullated fibres some 30 small medullated fibres were distinguishable. It will be seen that, if these are not

included, the difference in the totals is only four—such a difference as cannot be considered of any significance in view of the inaccuracy of the drawing method. If, however, the fibres are included, there is a difference of 34.

In all the measurements it will be seen that there is an excess in the roots of fibres of the larger classes—12μ and upwards. This is always practically balanced by an excess of the fibres between 6μ and 12μ in the trunk. Thus if we examine the number of fibres over 6μ in the three cases we find:

	Roots	Trunk
4th coccygeal	769	783
7th „	656	646
1st „	1151	1161

Here in one case there is an excess of 10 on the side of the roots, and in the other two differences of 14 and 10 on the side of the trunk. In every case, if the grey ramus is included, there is a preponderance of fibres less than 6μ in diameter in the trunk, as follows:

	Roots	Trunk
4th coccygeal	745	765
7th „	546	571
1st „	1254	1266

The result is thus always a preponderance on the side of a trunk. If, in the case of the 4th coccygeal, we omit the grey ramus fibres, we find an excess of 10 fibres under than 6μ in the roots, which nearly balances the excess of 14 fibres over 6μ in the trunk. It would be possible to interpret this as indicative of the presence of a small number of the unequal T divisions above referred to; but as it is often difficult to decide the class of a fibre which is near the limit, I do not think any such conclusion is justifiable, especially as the figures for the 7th coccygeal nerve show a very similar difference in the opposite direction.

The difference observed in the large fibres might be due either to greater shrinkage in the trunk, of which there is evidence in some of the sections, or to tapering of the fibres. That the latter process plays an important part I am inclined to believe on account of the following measurements, made in a precisely similar manner, of two sections of a ventral root, taken respectively near the cord and near the ganglion.

Diameter	Near the Cord		Near the Ganglion	
20μ & upwards	1		0	
19μ—20μ	1		1	
18μ—19μ	11		7	
17μ—18μ	16	215	12	200
16μ—17μ	18		21	
14μ—16μ	87		78	
12μ—14μ	81		81	
10μ—12μ	67		74	
8μ—10μ	24	126	22	128
6μ—8μ	35		32	
Less than 6μ	313		326	
	654		654	

Here it is evident that we have a difference of the same kind, though hardly so marked, as that seen in the other measurements.

In such circumstances it is difficult to draw conclusions as to the presence of unequal T divisions such as those described by Lenhossek and others (*vide sup.*), but there is, at any rate, no evidence for the general prevalence of such an arrangement, nor does it seem likely that the method could show any characteristic difference with regard to this matter in the various classes of fibres in the dorsal root. I have, therefore, not pursued the matter further.

Conclusions.

In the cat there are constantly a few more medullated fibres in a nerve immediately distal to a spinal ganglion than in the nerve immediately proximal to the ganglion, *i.e.* shortly, there are a few more medullated fibres in the trunk than in the nerve-roots. The excess is on an average about 0·5 p.c. of the total number of nerve-fibres. The results closely resemble those obtained by Holl, Stiénon, and Birge in other animals.

The excess of nerve-fibres in the trunk is apparently caused by fibres of not more than 6μ in diameter. The nerve-fibres which cause the excess are in all probability medullated fibres which pass to the trunk by way of the grey ramus communicans, and end in connection with the vessels or other tissue of the ganglion. That such fibres must

be taken into account has been suggested by Dogiel, Cajal and v. Gehuchten.

Allowing for these fibres, I find that the number of fibres close to the ganglion is the same as the number of fibres several millimetres from it both proximately and distally, *i.e.* none of the medullated fibres given off by the ganglion cells end in the nerve or roots close to the ganglion. This differs from the result obtained by Hardesty.

There are more fibres of 6μ and upwards in the anterior root close to the spinal cord than in the anterior root close to the ganglion, *i.e.* the fibres taper slightly.

There is a similar difference between the size of the nerve-fibres in the roots and that of those in the trunk. Probably then the posterior root-fibres also taper slightly.

The tapering of the motor fibres makes it impossible to draw definite conclusions as to the relative size of the central and peripheral processes of the spinal ganglion cells, but the evidence is against the view that the central processes are, in general, conspicuously smaller.

In conclusion I desire to express my indebtedness, for much kind advice and criticism, to Dr Langley, and to thank Dr H. K. Anderson for his ready help.

BIBLIOGRAPHY.

(1) Holl. Sitzungsberichte d. k. Akad. in Wien LXXII. Abth. 3, p. 31. 1875.
(2) Stiénon. Annales de l'Univ. libre de Bruxelles. 1880.
(3) Birge. Arch. f. Anat. und Physiol. Physiol. Abth. p. 435. 1882.
(4) Gaule and Lewin. Centralblatt für Physiologie, 1896, p. 437.
(5) Bühle. Verhandl. d. phys. med. Gesellsch. zu Würzburg. 1898.
(6) Hardesty. Journal of Comparative Neurology (Granville, Ohio). 1899.
(7) Ramon y Cajal. Arch. f. Anat. und Physiologie (Anat. Abth.) p. 319. 1893.
(8) Dogiel. Internationale Monatschrift f. Anat. und Physiolog. XIV. p. 73. 1897.
(9) Langley. Journal of Physiol. XX. p. 55. 1896.
(10) Wagner. Handwörterbuch d. Physiologie III. Abth. 1, pp. 362–366. 1846.

(11) Freud. Sitzungsberichte d. k. Akad. in Wien, LXXVIII. Abth. III. p. 81. 1878.
(12) Lenhossek. Arch. f. mikr. Anat. XXVI. p. 370. 1885.
(13) van Gehuchten. La Cellule. VII. p. 88. 1891.
(14) Bidder and Volkmann. Die Selbständigkeit des sympathischen Nervensystems. Leipzig. 1842.

PLATE II.

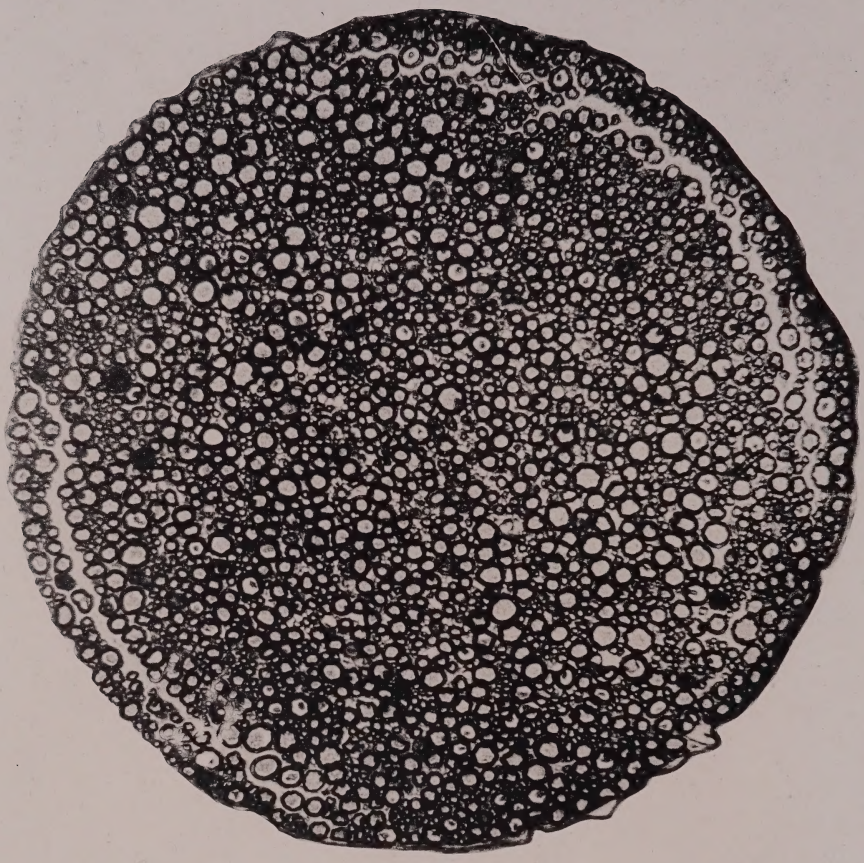
Specimens of the photographs used in counting.

Fig. 1. Trunk of 1st Coccygeal, Cat D.

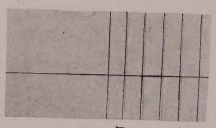
Figs. 2 and 3. Ventral Root of 1st Coccygeal, Cat D.

Figs. 4, 5, 6. Dorsal Root of 1st Coccygeal, Cat D.

The expenses of this research have been defrayed by a grant from the Government Grant Committee of the Royal Society.

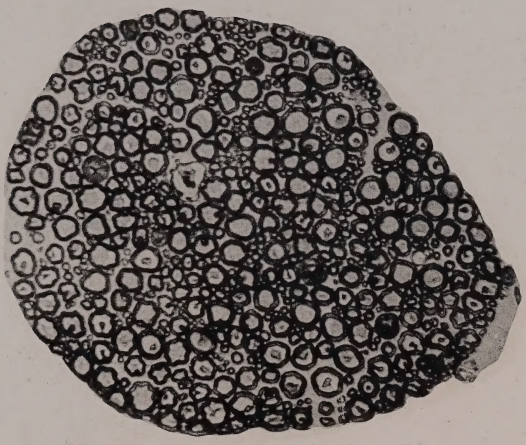


I

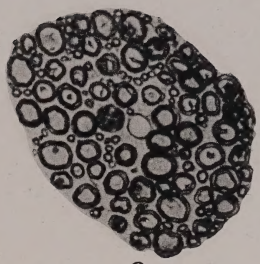


7

Division = $\frac{1}{100}$ mm.

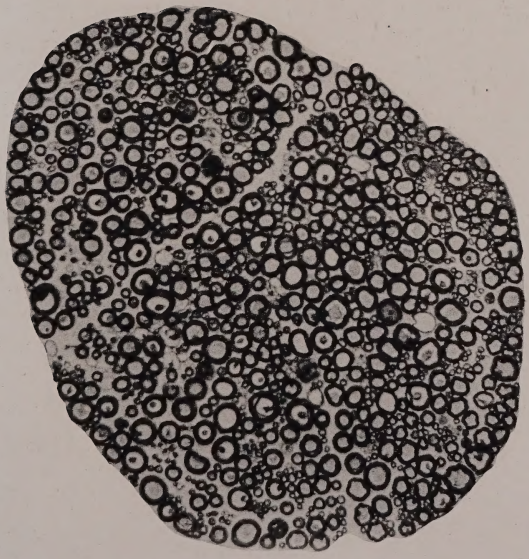


2

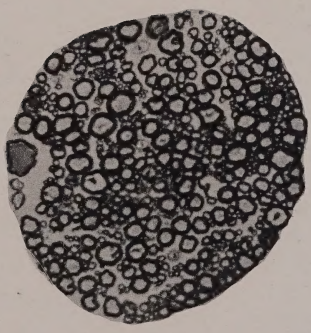


3

5



4



6